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The influence of thiazolidinediones on adipogenesis in vitro and in vivo: Potential modifiers of intramuscular adipose tissue deposition in meat animals^{1,2}

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ABSTRACT: Thiazolidinediones (TZD) are insulin sensitizing agents currently used for the treatment of type 2 diabetes and are widely used as adipogenic agents because they are ligands of peroxisome proliferator-activated receptor gamma (PPAR γ), a key adipogenic transcription factor. In vivo and in vitro studies of TZD as potential modifiers of intramuscular or marbling adipogenesis are reviewed. Thiazolidinedione-induced adipogenesis has been reported in numerous cell culture systems, including rodent, human, bovine, and porcine adipose tissue stromal-vascular (S-V) cell cultures. Studies of porcine S-V cell cultures derived from semitendinosus muscle show that TZD can potentially modify intramuscular or marbling adipogenesis. Preadipocyte recruitment was TZD-dependent in muscle S-V cultures but TZD-independent in adipose S-V cultures.

There appear to be differences between adipocytes in muscle and subcutaneous adipose tissue, reminiscent of differences observed in adipocytes from different adipose tissue depots. Troglitazone, a TZD, induces marbling adipogenesis without inhibiting myogenesis when cells are grown on laminin precoated culture dishes. Additionally, troglitazone treatment does not increase lipid content in porcine adipose tissue or muscle S-V cell cultures. Thiazolidinedione treatment increases lipid content of muscle in rodents and humans; however, rosiglitazone treatment for 49 d in pigs did not influence muscle lipid content and meat quality, but several significant changes in muscle fatty acid composition were observed. Although timing of treatment with TZD needs to be optimized, evidence suggests these compounds may enhance marbling deposition in swine.

Key words: thiazolidinedione, porcine, marbling, adipogenesis, skeletal muscle, adipose, CCAAT/enhancer binding protein alpha

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INTRODUCTION

Meat quality is adversely influenced by increased feed efficiency and lean tissue accretion induced by nutrition, genetic selection, or a number of dietary factors and metabolic modifiers (Van Barneveld, 2003; reviewed in Dunshea et al., 2005). In particular, increases in lean tissue accretion have resulted in decreased intramuscular adipose tissue deposition (Van Barneveld, 2003; re-

viewed in Dunshea et al., 2005; Schwab et al., 2006, 2007), which could reduce juiciness, flavor, and overall desirability of meat (reviewed in Wood et al., 1999). For instance, selection for leanness over the last 21 yr in purebred Duroc hogs has decreased intramuscular fat and adversely influenced pork flavor and color (Schwab et al., 2006). The continuing demand for increased marbling fat and meat quality has, therefore, generated many studies focused on intramuscular adipose tissue, also known as marbling fat, in an attempt to maintain or improve meat quality (reviewed in Van Barneveld, 2003; Dunshea et al., 2005). Studies have examined the influence of age, gender, dietary protein, various hormones, and muscle type on intramuscular adipose tissue or marbling accretion (reviewed in Poulos and Hausman, 2005). More recent studies indicate that marbling is influenced by steroyl-CoA desaturase protein expression (Doran et al., 2006), fatty acid binding protein (FABP)-4 protein levels (Damon et al., 2006), FABP genotype

¹Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

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(Michal et al., 2006), heart-FABP genotype (Pang et al., 2006), and IGF-II genotype (Gardan et al., 2006).

INTRAMUSCULAR/MARBLING PREADIPOCYTE DEVELOPMENT

The demand for maintaining marbling fat and meat quality has generated interest in agents or dietary constituents that could enhance or modify marbling deposition. Conjugated linoleic acid has been the most studied of potential modifiers of marbling deposition in pigs. For instance, dietary CLA increases marbling deposition while decreasing or maintaining subcutaneous fat deposition in growing pigs (Ostrowska et al., 1999; Wiegand et al., 2001, 2002; Tischendorf et al., 2002; Sun et al., 2004). Indirect evidence indicated that CLA supplementation of pig feed may induce the development or recruitment of intramuscular preadipocytes from stromal-vascular (S-V) cells (Meadus et al., 2002). However, intramuscular preadipocyte development, per se, was not examined since analysis was restricted to adipocyte marker gene expression in muscle samples (Meadus et al., 2002). Nevertheless, these studies indicate that preadipocyte development in subcutaneous and intramuscular depots may be regulated differently. Little is known about the growth and development of intramuscular adipocytes or the regulation of intramuscular adipogenesis. Furthermore, *in vitro* recruitment or developmental regulation of intramuscular preadipocytes from meat animals has, for the most part, not been examined. This lack of information limits the development of methods to regulate growth of these cells and improved marbling fat content of meat animals. Additionally, the early development of subcutaneous and intramuscular preadipocytes has never been compared despite the marked differences in the ontogeny and regulation of subcutaneous and intramuscular adipocyte cellularity in pigs (reviewed in Allen, 1976).

A collagenase digestion protocol for adipose tissue S-V cells was adapted to whole fetal and neonatal semitendinosus muscles (muscle-S-V cell cultures) to quantify the number of intramuscular preadipocytes and their response to adipogenic agents (Hausman and Poulos, 2004; 2005). Muscle S-V cells were rinsed 1 h after plating to reduce attachment of satellite cells and other myogenic cells (Hausman and Poulos, 2005). Some myogenic cells remain in culture despite rinsing, so myotube development was observed in muscle S-V cell cultures but only when S-V cells were plated on laminin substrata (Hausman and Poulos, 2005). Adipogenesis induced with dexamethasone (DEX), however, was independent of substrata in muscle S-V cultures (Hausman and Poulos, 2004). Plating muscle S-V cells on laminin allowed comparison of the response of preadipocytes and myotubes developing in the same culture to adipocyte differentiation inducing agents. Expression of nuclear peroxisome proliferator-activated receptor (PPAR)- γ protein was restricted to lipid accreting preadipocytes in muscle S-V cultures regardless of substrata (Hausman and Poulos,

2005). Preadipocyte lipid accretion was associated with expression of nuclear PPAR γ protein in muscle and adipose S-V cultures (Hausman and Poulos, 2004). Dexamethasone induced considerably less adipogenesis in muscle S-V cultures than in adipose tissue S-V cultures (Hausman and Poulos, 2004). This discrepancy was, in part, attributable to a lower number of preadipocytes in muscle S-V cultures from the onset of culture (Hausman and Poulos, 2004). These studies indicate that inherent differences exist between intramuscular preadipocytes and preadipocytes of other depots. These differences could allow for the controlled increase in marbling fat without a concomitant increase in other fat depots, including subcutaneous fat, which are not desirable.

THIAZOLIDINEDIONES AND GLITAZONES

For many reasons, orally administered thiazolidinediones (TZD) represent a potential means to increase intramuscular adipose tissue or marbling deposition. Thiazolidinediones are PPAR γ ligands that are used clinically as insulin sensitizing agents for the treatment and control of type 2 diabetes (reviewed in Reynolds and Goldberg, 2006). Troglitazone, rosiglitazone, and pioglitazone are members of the TZD class of antidiabetic agents proven to be effective clinically (Lebovitz, 2002). Although the TZD are not currently approved for use in meat animals, there are potentially great economic advantages of using these compounds. The significant cost of developing a new compound has already been invested by pharmaceutical companies for human health. There is likely a cost benefit to pharmaceutical companies in providing existing compounds for alternative industries, implying these agents could be sold at significantly lower prices than agents developed specifically for one industry.

Despite a common mode of action, troglitazone, rosiglitazone, and pioglitazone have unique chemical structures and receptor binding affinities and side effects (Lebovitz, 2002). Additionally, TZD are widely used to induce differentiation of preadipocytes, via activation or expression of PPAR γ , in a wide variety of cell culture systems (Table 1). Studies of PPAR γ knockout mice and cells demonstrate that activation and expression of PPAR γ , particularly, PPAR γ 2 is key to adipose tissue development *in vivo* and *in vitro* (Rosen et al., 1999; Zhang et al., 2004). A study of several nonTZD PPAR γ agonists, a TZD PPAR γ agonist (AD-5075), a PPAR α agonist, and a PPAR δ agonist showed that the PPAR γ binding affinity of PPAR agonists dictated their effectiveness *in vivo* and *in vitro* (Berger et al., 1999; Figures 1 and 2). For instance, TZD and nonTZD PPAR γ agonists induced adipogenesis in 3T3-L1 cultures (Figure 1) and normalized glucose and triglyceride levels in diabetic mice to a much greater degree than PPAR δ (L-165041) or PPAR α (WY-14543) agonists (Berger et al., 1999). Plotting the ED₅₀ (ED = effective dose) for normalizing blood glucose levels against PPAR γ binding affinities confirms the importance of PPAR γ binding (Figure 2).

Table 1. The influence of thiazolidinediones (TZD) on various adipogenic cells in vitro¹

Cell type	TZD influence	References
Established readipocyte lines	Various TZD induce adipogenesis via PPAR γ gene expression in many preadipocyte cell culture systems	Reviews, Sharma and Staels, 2007; Reynolds and Goldberg, 2006
Primary pig and fetal pig adipose tissue S-V cells	Troglitazone and ciglitazone induce preadipocyte recruitment and differentiation with PPAR γ and C/EBP α expression but with little lipid accretion	Tchoukalova et al., 2000; Poulos and Hausman, 2006a,b
Primary pig muscle S-V cells	Troglitazone induces preadipocyte recruitment and differentiation (PPAR γ and C/EBP α expression) with little lipid accretion	Poulos and Hausman, 2006b
Primary bovine adipose tissue S-V cells	Troglitazone, T-174, DEX, and IBMX induce adipogenesis with PPAR γ and C/EBP α expression	Ohyama et al., 1998; Hirai et al., 2006, 2007; Soliman et al., 2006
Primary and secondary human adipose tissue S-V cells	Troglitazone or rosiglitazone induce adipogenesis with PPAR γ and C/EBP α expression but with little lipid accretion	Hutley et al., 2003; Tomlinson et al., 2006
Primary bovine fibroblast-like cells	T-174 or Wy14,643 induce adipogenesis in muscle associated fibroblasts expressing PPAR γ	Torii et al., 1998
Porcine, bovine, and human satellite cells	Ciglitazone or rosiglitazone \pm DEX and IBMX, induce adipogenesis with PPAR γ and C/EBP α expression	DeCoppi et al., 2006; Kook et al., 2006; Singh et al., 2007

¹C/EBP α = CCAAT/enhancer binding protein α ; DEX = dexamethasone; IBMX = 3-isobutyl-1-methylxanthine; PPAR γ = peroxisome proliferator-activated receptor γ ; and S-V = stromal-vascular.

Recent studies have demonstrated that TZD influences many cell types including inducing adipogenesis in satellite cells and fibroblast-like cells from muscle (Table 1), increasing proliferation, migration, and secretions from endothelial cells (Fukunaga et al., 2001; Pistrosch et al., 2005), inhibiting monocyte migration (Tanaka et al.,

2005), and inducing apoptosis in macrophages (Bodles et al., 2006).

THIAZOLIDINEDIONES: IN VITRO STUDIES

Studies of primary adipose tissue S-V cell cultures demonstrate the potential of TZD to induce adipogenesis, alone or in combination with DEX (Table 1). Troglitazone and DEX, alone or in combination, induced preadipocyte recruitment and differentiation similarly in porcine adipose tissue S-V cultures (Table 1). The ability to recruit preadipocytes indicates that TZD influence a very early stage of preadipocyte commitment (Hausman and Richardson, 1998). Troglitazone induced adipogenesis in fetal

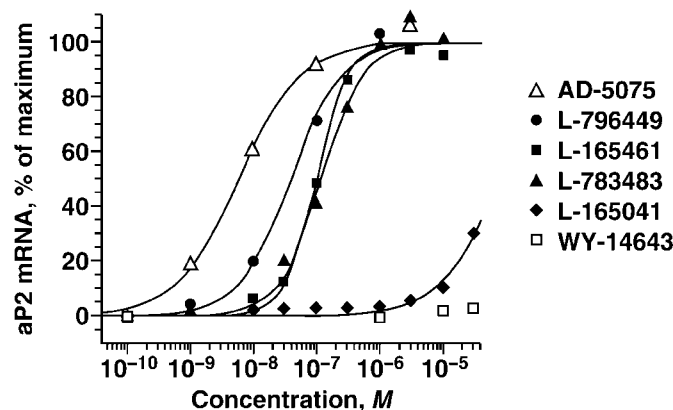


Figure 1. Expression of aP2 mRNA in confluent 3T3-L1 preadipocytes that were incubated with the indicated concentrations of peroxisome proliferator-activated receptor γ (PPAR γ) ligands (AD-5075, L-796449, L-165461, and L-783483), peroxisome proliferator-activated receptor δ (PPAR δ) ligand (L-165041), or peroxisome proliferator-activated receptor α (PPAR α) ligand (WY-14643) for 4 d. The only thiazolidinedione (TZD) PPAR γ ligand used was AD-5075. Expression of aP2 mRNA indicates preadipocyte differentiation because aP2 is induced and increases with preadipocyte differentiation (from Berger et al., 1999; with permission of The American Society for Biochemistry and Molecular Biology Inc.).

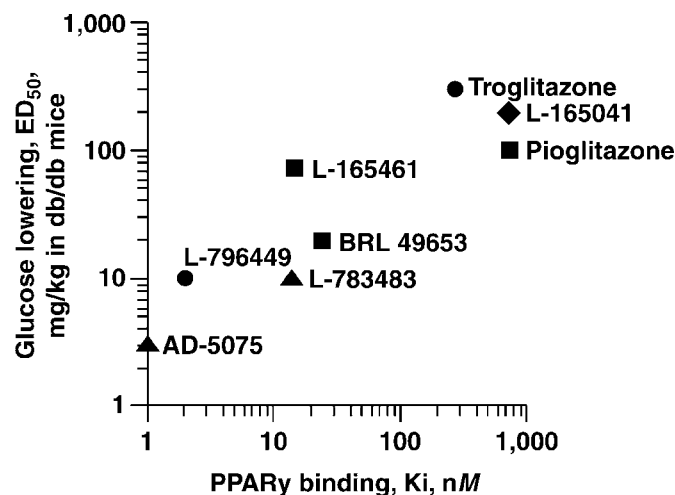


Figure 2. Approximate ED₅₀ values for glucose lowering in db/db mice vs. K_i values determined for peroxisome proliferator-activated receptor γ (PPAR γ) binding.

Table 2. Influence of chronic thiazolidinedione (TZD) treatment in vivo on adipose tissue and muscle¹

Species and tissue	TZD influence	References
Human adipose tissue	Induce adipogenesis via PPAR γ gene expression	Tiikkainen et al., 2004; Kolak et al., 2007
Human and rat adipose tissue	Depot specific remodeling: smaller and clustered adipocytes, decreased inflammation, and increased lipid storage	Okuno et al., 1998; de Souza et al., 2001; Berthiaume et al., 2004
Human adipose tissue	Promote mitochondrial biogenesis and oxidative pathways via PGC-1 α upregulation	Bogacka et al., 2005; Hondares et al., 2006
Human, rat, and mouse muscle	Increased glucose uptake and adipocyte gene expression	Reviews, Poulos and Hausman, 2005; Reynolds and Goldberg, 2006
Rat, mouse, and monkey muscle	Increased glycogen deposition	Burant et al., 1997; Oshida et al., 1999; Ortmeier et al., 2000

¹PGC-1 α , PPAR γ coactivator-1 α , and PPAR γ , peroxisome proliferator-activated receptor γ .

pig adipose tissue S-V cultures earlier than DEX (Poulos and Hausman, 2006a). Lipid accretion, however, was not influenced by TZD in pig muscle S-V cultures (Poulos and Hausman, 2006a,b) and in human (Hutley et al., 2003; Tomlinson et al., 2006), fetal pig (Poulos and Hausman, 2006a), and pig adipose tissue S-V cultures (Tchoukalova et al., 2000; Poulos and Hausman, 2006b; Table 1). In contrast, lipid accretion in human adipose tissue S-V cultures was markedly dependent on the presence of TZD in media with high levels of DEX and insulin (Brown et al., 2001). Dose-response curves in adipose tissue and muscle S-V cultures demonstrated the TZD, troglitazone, and ciglitazone, induced greater preadipocyte recruitment in S-V cells from adipose tissue than from skeletal muscle (Poulos and Hausman, 2006b; Table 1). However, TZD enhanced expression of other adipogenic markers, such as C/EBP α protein expression, similarly in adipose tissue and muscle S-V cultures (Table 1). Troglitazone failed to influence preadipocyte recruitment and adipogenesis in fetal pig muscle S-V cultures (Poulos and Hausman, 2006a). Therefore, PPAR γ content or affinity for TZD may distinguish fetal and young pig muscle preadipocytes from adipose tissue preadipocytes. Some of the adipogenic cells in muscle S-V cultures may be satellite cells because TZD induce adipogenesis in muscle satellite cell cultures (DeCoppi et al., 2006; Kook et al., 2006; Singh et al., 2007; Table 1).

THIAZOLIDINEDIONES: IN VIVO STUDIES

The influence of TZD treatment on muscle and adipose tissue has been studied primarily in humans, rodents, and monkeys with no such studies reported in the bovine or porcine species. Studies in pigs have been limited to cardiovascular studies of TZD-treated neonatal pigs (Zhu et al., 2000; Xu et al., 2005). Chronic TZD treatment in vivo increases adipogenic gene expression and the number of small adipocytes in adipose tissue which results in a remodeled morphology (Table 2). Recent studies indicate that TZD can also enhance gene expression in adipose tissue related to mitochondrial biogenesis (Bogacka et al., 2005; Hondares et al., 2006; Table 2). The insulin sensitizing capability of TZD is attributable, in part, to increasing muscle glucose uptake and glycogen deposition (Burant et al., 1997; Oshida et al., 1999; Ort-

meyer et al., 2000; Poulos and Hausman, 2005; Reynolds and Goldberg, 2006; Table 2). The influence of rosiglitazone on intra and extramuscular lipid accretion is variable and dependent on species (Table 3). Increased intramuscular lipid accumulation has been observed in rosiglitazone-treated rodents and humans (Mayerson et al., 2002; Muurling et al., 2003; Lessard et al., 2004; Table 3). Additionally, a decrease in the intramyocellular to extramyocellular ratio indicated lipid accumulation within intramuscular adipocytes was increased in Zucker rats treated with rosiglitazone (Lessard et al., 2004; Table 3). These studies indicate that rosiglitazone's action includes redistributing intracellular lipid from insulin responsive organs, like muscle, into adipocytes (Mayerson et al., 2002). Alternatively, rosiglitazone may increase muscle oxidative capacity independent of changes in intramyocellular lipid content (Mensink et al., 2007).

Treating pigs with rosiglitazone for 49 d before slaughter did not influence muscle lipid content, as assessed by lipid extraction, or meat quality traits but did influence lipid accretion within semitendinosus muscle (S. P. Poulos, T. D. Pringle, M. J. Azain, and G. J. Hausman, unpublished results; Tables 3 and 4). Therefore, rosiglitazone may redistribute intracellular lipid away from muscle in pigs as in other species. In contrast, studies of Zucker rats treated with pioglitazone for 28 d indicated expression of the adipogenic genes (fatty acid synthase and phosphoenolpyruvate kinase) was increased in oxidative, soleus muscle, but not in extensor digitorum longus or epitrochlearis muscles (Hallakou et al., 1998). Interestingly, the soleus muscle was the only muscle that expressed significant amounts of fatty acid synthase before pioglitazone treatment (Hallakou et al., 1998). The authors suggested that TZD-induced differentiation of preadipocytes that were present before treatment and did not promote the transdifferentiation of myocytes into adipocytes, as has been previously described in vitro (Kausch et al., 2001).

Rosiglitazone influenced lipid staining and NADH-tetrazolium reductase reactivity in the deep and superficial portions of the semitendinosus muscle from growing pigs (Table 3, Figure 3). Rosiglitazone increased peripheral NADH (Figure 3) and lipid around the periphery of muscle fibers, which may indicate increased metabolic activ-

Table 3. The influence of thiazolidinedione (TZD) treatment on intra- and extramuscular triglyceride and fatty acid content and extramuscular fibroblasts¹

Type of Thiazolidinedione	Species and treatment duration	TZD influence	References
Rosiglitazone	Zucker fatty rats, 28 or 120 d	Reduction in intramyocellular to extramyocellular ratio in tibialis anterior muscle	Jucker et al., 2003;
Rosiglitazone	ob/ob mice, 70 d	Triglyceride content increased in skeletal muscle but decreased in cardiac muscle	Kuhlmann et al., 2003 Muurling et al., 2003
Rosiglitazone	Diabetic humans, 90 d	Increased extramyocellular lipid content	Mayerson et al., 2002
Rosiglitazone	Diabetic humans, 56 d	No change in intramyocellular lipid	Mensink et al., 2007
Rosiglitazone	Zucker rats, 42 d	Increased intramuscular triacylglycerol and palmitoleate content in fatty rats	Lessard et al., 2004
Rosiglitazone	Pigs, 49 d	Decreased lipid in deep semitendinosus muscle; no influence on carcass and meat quality	S. P. Poulos, T. D. Pringle, M. J. Azain, and G. J. Hausman, unpublished results
Ciglitazone	Mice, 3 d	Increased numbers of PPAR γ expressing fibroblasts adjacent to muscle	Lohrke et al., 2000

¹PPAR γ = peroxisome proliferator-activated receptor γ .

ity in those muscle fibers. Recent studies indicate that TZD may inhibit electron transport via inhibition of NADH reductase and dehydrogenase activities (Jové et al., 2004; Scatena et al., 2004). This inhibition could result in NADH accumulation and may explain the increased NADH reactivity observed in this study (Figure 3). Additionally, TZD-induced NADH accumulation may suppress β -oxidation with a concomitant increase in glucose uptake, glycolysis, and lipid accumulation in diabetic animals and humans (Scatena et al., 2004). Although muscles from diabetic patients have decreased mitochondrial contents, TZD treatment does not alter muscle fiber type (Mathieu-Costello et al., 2003). In vitro studies have shown that TZD induce adipogenesis (Table 1) and the expression of several adipose tissue genes, plasma membrane fatty-acid-binding protein (**FABPm**), adipocyte fatty acid-binding protein (**aP2**, **FABP4**), per-

oxisome proliferator-activated receptor γ 2 (**PPAR γ 2**), and glycerol-3-phosphate dehydrogenase (**GPDH**; Kausch et al., 2001).

Rosiglitazone treatment alters fatty acid composition in rodents, and to a lesser extent, in porcine superficial semitendinosus and longissimus dorsi muscles (Table 4). Darglitazone reduced the ratio of unsaturated to saturated fatty acids in mouse brown adipose tissue, primarily because of an increase in palmitoleic acid (Aleo et al., 2003). Furthermore, skeletal muscle lipid composition was more unsaturated, which was also due to increased palmitoleic acid, in Zucker rats following 6 wk of rosiglitazone treatment (Lessard et al., 2004). The changes in fatty acid composition of muscle and adipose tissue may be due to decreased Δ 6 desaturase activity as troglitazone reduced Δ 6 desaturase gene expression in human skeletal muscle cells (Wahl et al., 2002).

Table 4. Effect of rosiglitazone maleate treatment (0, 4, or 12 mg) on fatty acid composition (g/100 g of lipid) of longissimus muscle and superficial semitendinosus muscle samples collected from slaughter weight gilts after 49 d of treatment¹

Item	Longissimus muscle, mg				Superficial semitendinosus muscle, mg			
	0	4	12	Pooled SEM	0	4	12	Pooled SEM
Lipid content, g	12.96	10.68	11.84	1.76	14.2	15.6	14.8	2.0
Lipid type	g/100 g of lipid							
14:0	0.04	0.06	0.02	0.02	0.02	0.04	0.02	0.01
14:1	0.05	0.04	0.09 ^b	0.01	0.05	0.06	0.06	0.005
16:0	0.78	1.03	0.55	0.44	0.43	0.94	0.57	0.20
16:1	0.09	0.13	0.04	0.05	0.05	0.12	0.06	0.02
18:0	0.47	0.57	0.29	0.21	0.24	0.48	0.33	0.10
18:1	1.42	2.05	0.86	1.03	0.51	1.97 ^a	1.04	0.43
18:2	0.47	0.53	0.50	0.24	0.39	0.61	0.49	0.08
18:3	0.01	0.01	0.00	0.009	0.01	0.01	0.01	0.003
20:4	0.08	0.07	0.17	0.02	0.01	0.01	0.03	0.01

^aDifferent from tissue derived from animals not treated with rosiglitazone ($P < 0.05$).

^bDifferent from tissue derived from animals treated with 4 mg of rosiglitazone ($P < 0.05$).

¹The percent of individual fatty acids constituting the total measurable fatty acid pool in samples of muscle. Values are least squares means ($n = 7$ to 8).

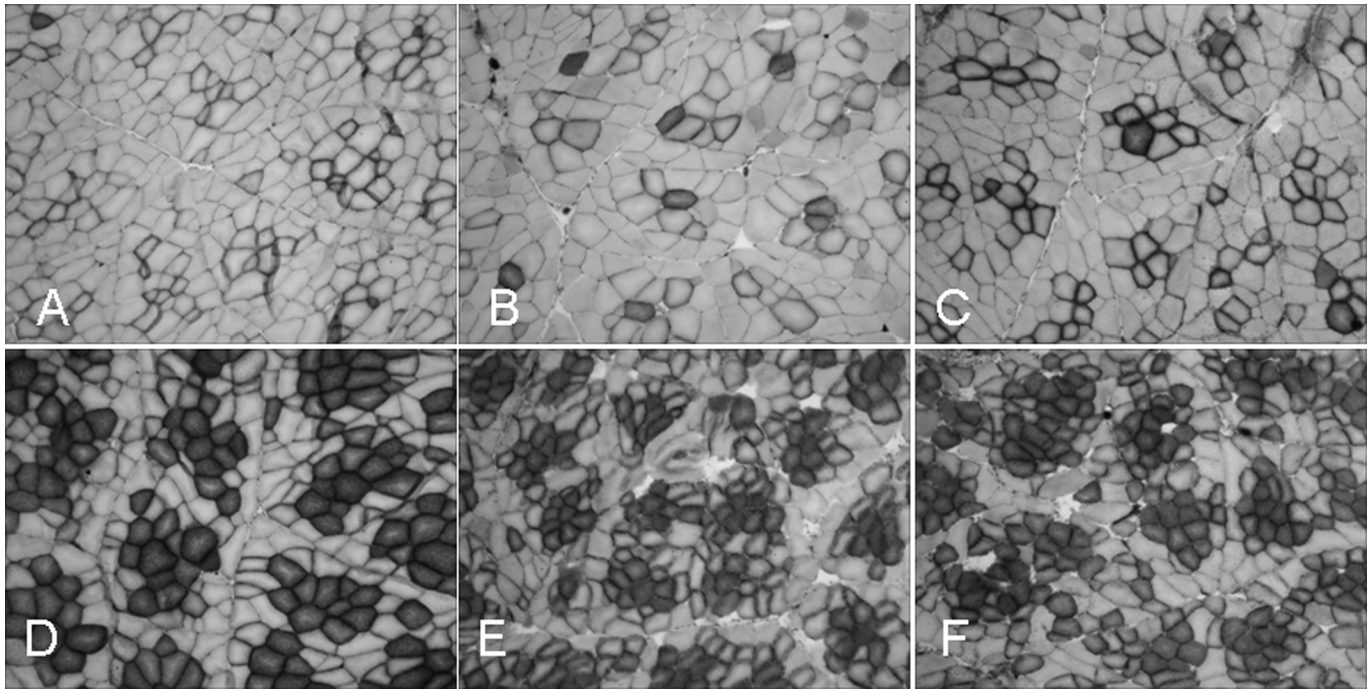


Figure 3. Staining of NADH-tetrazolium reductase (NADH-TR) in semitendinosus muscle sections from pigs treated with rosiglitazone for 49 d. Rosiglitazone increased NADH-TR reactivity in the periphery of muscle fibers in a dose-dependent manner in the superficial aspect. Sections of deep semitendinosus muscle are shown in panels A, B, and C, and superficial semitendinosus muscle are shown in panels D, E, and F, after treatment with 0 (A, D), 4 (B, E), or 12 (C, F) mg of rosiglitazone.

SUMMARY AND CONCLUSIONS

In summary, *in vitro* and *in vivo* studies indicate the potential of TZD as modifiers of marbling or intramuscular adipose tissue deposition in meat animals. Additionally, an initial *in vivo* study suggests this may be accomplished without compromising growth, meat quality, or carcass composition (Table 3). However, rosiglitazone appears to cause a shift in location of NADH and lipid to the periphery of muscle fibers, suggesting this compound may influence substrate metabolism in skeletal muscle. Nonetheless, the potential to alter energy metabolism in skeletal muscle using TZD in pigs should be further investigated. Studies to determine optimal timing for increased intramuscular adipogenesis, including possible treatment during the grower phase instead of during the finisher phase, should be investigated. Finally, evaluation of human medications for alternative use in animals, and the interpretation of human clinical trials for potential application in animal health or management may provide effective ways to improve health and management of animals.

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